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EFFECT OF ELECTROPORATION ON MICROBIAL CELL WALL TO EXTRACT LIPID

UMI RABIATUL RAMZILAH BT P.REMLI

Thesis submitted in fulfilment of the requirements
for the award of the degree of
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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of degree of Bachelor of Engineering Technology Energy & Environment

Signature:
Name of Supervisor: DR. ABU YOUSUF
Position: LECTURER, FACULTY OF ENGINEERING TECHNOLOGY
UNIVERSITI MALAYSIA PAHANG
Date: JANUARY 2016

Signature:
Name of Supervisor: ASSOC. PROF. DR. CHE KU FAIZAL BIN CHE KU YAHYA
Position: LECTURER, FACULTY OF ENGINEERING TECHNOLOGY
UNIVERSITI MALAYSIA PAHANG
Date: JANUARY 2016

Signature:
Name of Supervisor: DR. ABU YOUSUF
Position: LECTURER, FACULTY OF ENGINEERING TECHNOLOGY
UNIVERSITI MALAYSIA PAHANG
Date: JANUARY 2016

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries in which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature:

Name: UMI RABIATUL RAMZILAH BT P.REMLI

ID Number: TC 13026

Date: 12/1/2017

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ABSTRACT

Nowadays, fossil fuels are still the primary energy sources but it cannot hold for a lifetime. Therefore, to overcome this limitation, biofuel is considered as a sustainable solution. A number of methods have been developed for producing biodiesel but with traditional method its take more time and associated with usage of huge amount of toxic solvents. Therefore, our research aimed how to produce biodiesel in a short time, with less energy input and eco-friendly. To do this we used electroporation process, where we employed a high voltage for shorter time on yeast cell to disrupt them. Our reactor took around 10 minutes to disrupt the cell wall for escaping intracellular lipid which could be used for biodiesel synthesis. Then, from the electroporation, we can extract and quantify the lipids for further applications. Lipid extraction and quantification is the accurate and faster method to determine the lipid content that involve cell wall breakup and extraction with organic solvent. In lipid quantification, the result shown that, lipid produced is higher when treated with high pulse electric field at 10 minutes with 2 cm distance. This is due to the lower distance between the electrodes that influenced the intensity of the voltage applied to the treatment media. Furthermore, the longer the treatment time, more microbial cell wall was disrupt causing more intracellular lipids to come out to the surface. Finally, the potential of biofuels as one of energy source cannot be overlooked from the result of the present research because they can contribute considerably to supply energy for future.

ABSTRAK

Kini, bahan api fosil masih menjadi sumber tenaga utama tetapi ia tidak boleh bertahan lama. Oleh itu, untuk mengatasi masalah ini, bahan api bio dianggap sebagai penyelesaian yang terbaik. Beberapa kaedah telah dibangunkan untuk menghasilkan biodiesel tetapi dengan kaedah tradisional ia mengambil banyak masa dan menggunakan pelarut toksik yang banyak. Oleh itu, penyelidikan kami bertumpu kepada bagaimana untuk menghasilkan biodiesel dalam masa yang singkat, dengan input tenaga yang kurang dan mesra alam. Untuk melakukan ini kami menggunakan proses elektroporasi, di mana voltan yang tinggi digunakan untuk jangka masa yang singkat pada sel yis bagi memecahkan dinding sel tersebut. Reaktor kami mengambil masa 10 minit untuk memecahkan dinding sel untuk mengekstrak intraselular lemak yang boleh digunakan untuk sintesis biodiesel. Pengekstrakan dan kuantifikasi lemak adalah kaedah yang tepat dan cepat untuk menentukan kandungan lemak yang melibatkan perpecahan sel dinding dan pengekstrakan menggunakan pelarut organik. Dalam kuantifikasi lemak, keputusan menunjukkan bahawa, kandungan lemak yang terhasil adalah lebih tinggi apabila dirawat dengan elektrik berfrekuensi tinggi pada 10 minit dengan jarak 2 cm. Ini adalah kerana jarak yang lebih rendah antara elektrod akan mempengaruhi intensiti voltan yang digunakan untuk media rawatan. Tambahan pula, semakin lama masa rawatan, dinding sel mikrob yang telah pecah menyebabkan lebih banyak intraselular lemak keluar ke permukaan. Akhir sekali, potensi bahan api bio sebagai salah satu sumber tenaga yang tidak boleh diabaikan kerana ia boleh memberikan sumbangan yang besar untuk membekalkan tenaga pada masa depan.

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LIST OF SYMBOLS

cm	Centimeter
$CHCl_3$	Chloroform
CH_3OH	Methanol
°C	Degree Celsius
g	Gram
Hz	Hertz
kV	Kilovolt
m	Meter
µg	Micro gram
µl	Micro Liter
mg	Milligrams
ml	Milliliter
min	Minute

LIST OF ABBREVIATIONS

AC	Alternating Current
CFU	Colony Forming Unit
DC	Direct Current
DNA	Deoxyribonucleic Acid
DTE	Direct Transesterification
EP	Electroporation
FESEM	Field Emission Scanning Electron Microscope
PEF	Pulse Electric Field
RNA	Ribonucleic Acid

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Fossil fuel such as coal and oil plays a critical role in humanity history. These fuels are still the primary energy sources but it cannot hold for a lifetime. Eventually, these energy sources will be depleted someday according to many energy experts. Experts predicted that the rate of world oil production is already achieving its peak while the reserves are slowly depleted. Not only that the demand for fossil fuel keep increasing especially from the developing country. This decreasing supply of fuel and rising demand is not good to consumer as the prices of oil will keep increase year by year. This can make the economy to be unsustainable. Furthermore, burning of the fossil fuel also contribute to the greenhouse effect as the emission of CO2 mainly from transportation sector and industry. These will contribute more to global warming which can lead to climate change. Climate change can have a negative impacts towards all life in the earth.

To overcome these scenarios, biofuel is considered as a sustainable solution. Biodiesel, a promising renewable liquid biofuel, has been deliberated as an alternative of petroleum based fuel. High production cost attributed to feedstock is the main obstacle to its wide application (Yousuf 2012). Presently biodiesel is produced from vegetable oils, waste cooking oils and nonedible plant oils. Vegetable oils like rapeseed oil, sunflower, corn oil, canola oil, rice bran oil contribute to the world's food supply, therefore, use of them as biodiesel feedstock arise a food versus fuel conflict (Adamczak et al. 2009).

On the other hand, waste cooking oil is not feasible because of its limited supply. Microbial oils or lipids, which are produced by oleaginous microbial community such as microalgae, yeast and fungi, have been considered as a promising alternative of vegetable oils for biodiesel synthesis. However, high fermentation cost limits their large scale production (Yousuf et al. 2010). Some inexpensive medium have been suggested to reduce the fermentation cost such as industrial wastewater (Cheirsilp and Louhasakul 2013; Yousuf et al. 2010), industrial glycerol (Papanikolaou and Aggelis 2009), molasses (Zhu et al. 2008) and lignocellulosic hydrolysates (Yousuf 2012).

Although low cost medium have been developed, the processing of microbial oils is still a challenging task. The common processing steps are microbial biomass drying, cell disruption, oil extraction, separation and esterification (Cheirsilp and Louhasakul 2013). These steps are associated with high temperature, long processing time, addition of huge solvents and high financial matters. Therefore, direct transesterification (DTE) has been proposed which eliminates biomass drying, lipid extraction and separation steps. According to this process, esterification is carried out directly with wet biomass which substantially reduce the time, solvent use and ultimately the production cost of biodiesel.

However, water content of biomass makes slower the esterification reaction and lipid transfer from microbial cell to reaction medium become inefficient because of bulk reaction volume. Therefore, the present study proposes an innovative solution to overcome these shortcomings of DTE of microbial oils.

1.2 PROBLEM STATEMENT

Though DTE offer shorter processing time and lower production cost of biodiesel from microbial biomass, still need to be developed some factors to attain potential biodiesel production. Such as:

1.2.1 Cell Wall Breakup

The main difficulties that have to face in DTE, is the disruption of microbial cell wall. The yeast cell wall is basically a rigid but adaptable polysaccharide/proteinaciouscage, which confers both cell integrity and cell shape, and is essential for survival in natural environments, i.e. in media devoid of osmotic stabilization (Backhaus et al. 2013). The layer between cell surface and hydrophobic solvents prevents lipid extraction.

1.2.2 Water Content

Water levels of microbial biomass significantly influence the production costs of biodiesel by DTE process. If microbial biomass contain higher amount of water, it decreases the extraction efficiency of intracellular lipid (Hidalgo et al. 2013). In that case, it requires a higher molar ratio of alcohol to total lipid content for its in-situ transesterification. If dried biomass is used as feed stock, it consumes less solvent, process become faster, and results in higher biodiesel yields (Kakkad et al. 2015). Therefore, it is essential to overcome this limitations to get benefits from the in-situ transesterification.

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